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EDITORIAL COMMENT

"Positive Contrast" Inversion-Recovery With Oxide Nanoparticles-Resonant Water Suppression Magnetic Resonance Imaging

A Change for the Better?*

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The identification and characterization of atherosclerotic lesions prone to rupture in asymptomatic individuals remains a major focus of diagnostic imaging research. Although ischemic events often result from severely stenotic lesions in the peripheral arteries, the majority of atherosclerotic lesions responsible for acute coronary syndromes are typically <50% stenosed prior to the event (1,2). Studies have clearly shown that vulnerability is dependent on plaque composition, with most vulnerable plaques exhibiting high macrophage density, lipid rich cores, necrosis, and the establishment of plaque neovasculature (3). This may explain why over one-half of individuals present with myocardial infarction or sudden death as their first manifestation of atherosclerosis (4). Development of noninvasive molecular imaging probes that target components of vulnerable atherosclerotic plaque may enable improved detection of lesions prone to rupture and may allow for early therapeutic intervention (5). However, this raises the larger question of whether imaging can provide information in addition to proven clinical risk factors that may alter therapy other than simply initiating cardioprotective medications.

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High-resolution magnetic resonance imaging (MRI) has emerged as a leading noninvasive imaging modality for characterizing atherosclerotic plaque in vivo (6). Magnetic resonance imaging provides high-spatial-resolution images without the need for ionizing radiation and can be repeated sequentially over time to monitor therapeutic intervention or assess plaque progression and regression. Due to the importance of macrophages in plaque progression and destabilization, several paramagnetic and superparamagnetic MRI agents have been developed to specifically image the macrophage burden in atherosclerotic plaque (7,8). Although macrophage targeting using gadolinium-based nanoparticles appears promising (9), the safety of these agents has yet to be established. Iron oxide particles, on the other hand, have long been used clinically to target cells associated with both the reticular endothelial system and monophagocytic system (10).

Iron oxide particles range in size from ultrasmall superparamagnetic particles of iron oxide (SPIO) with a diameter of ~20 nm to SPIO with a diameter of ~100 nm to microparticles of iron oxide with a diameter of 1 μm. These particles consist of an iron oxide core(s) and sometimes a coating material such as dextran. Cross-linked iron oxide nanoparticles have also been modified with other imaging probes to generate dual-imaging agents that enable both magnetic resonance (MR) and optical in vivo imaging of specific targets or cellular enzymatic activity (11). Low toxicity, commercial availability, and the ability to modify particle size and coating (affecting circulation time, cellular uptake, and plaque penetration) are advantages of these contrast agents. Generally, for particles with similar coating, uptake and therefore MR efficacy increases as the particle size decreases (12).

The contrast agents appear to be phagocytosed by mononuclear cells, enabling quantification of resident macrophage burden and the degree of inflammation in atherosclerotic plaque (13). Internalization and compartmentalization of iron oxide particles within macrophages causes MR signal loss due to the generation of local magnetic field inhomogeneities that induce T_2/T_2^* effects (14). Typically, T_2 and/or T_2^*-weighted sequences are used to observe the signal loss generated after the uptake of the iron oxide particles within the cells. Iron oxide nanoparticles can also be modified with antibodies to image specific ligands in atherosclerotic plaques (15). In vivo MRI of stem cell (16) or inflammatory cell (17) trafficking has also been achieved by extracting the cells of interest and "loading" them with iron oxide particles to characterize their migration into atherosclerotic lesions or their role in other disease processes. Figure 1 illustrates different strategies of identifying atherosclerotic plaque and degree of inflammation using iron oxide particles.

However, interpretation of the signal loss generated by the particles may be challenging due to issues associated

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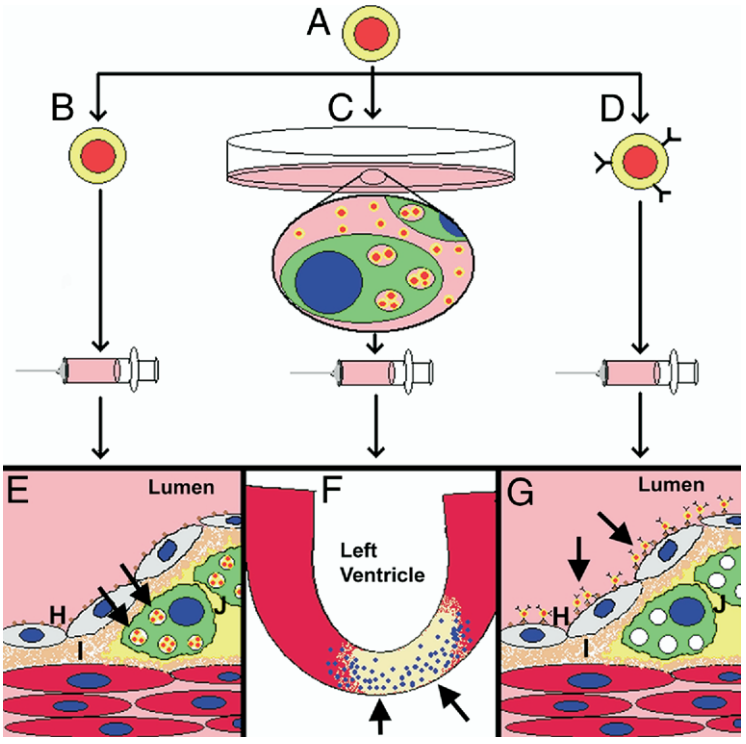

Figure 1 **3 Imaging Strategies Using Iron Oxide Nanoparticles**

An illustration of 3 strategies using iron oxide nanoparticles **(A)** for imaging the cardiovascular system with positive contrast inversion-recovery with oxide nanoparticles–resonant water repression magnetic resonance imaging (MRI). Iron oxide particles alone **(B)** can be injected intravenously and, as seen in **panel E**, iron oxide particles **(arrows)** pass through the endothelium **(H)**, fibrous cap **(I)**, and are taken up by resident macrophages in the lipid core **(J)**. Alternatively, stem cells can be incubated with and take up iron oxide particles **(C)**. The cells can then be injected intravenously and cellular migration can be determined with MRI. As seen in **panel F**, MRI may be used to detect stem cells loaded with iron oxide particles **(arrows)** that migrate to a region of myocardial infarction. Finally, iron oxide nanoparticles can be attached to antibodies that specifically target components of atherosclerotic plaque **(D)**. As seen in **panel G**, MRI can be used to detect integrins expressed on the surface of endothelial cells **(H)** with iron oxide particles attached to antibodies targeting vascular cell adhesion molecule-1 or other integrins **(arrows)**.

with partial voluming, perivascular effects, and other susceptibility artifacts. It is often challenging to distinguish the signal void generated by the contrast agents from motion or flow artifacts and inherent T_2^* effects in the plaque. Signal loss is also heavily dependent on image resolution and regional contrast agent concentration. To overcome these limitations, several groups have developed "positive" contrast imaging sequences as an alternative means of detecting iron oxide particles with MRI (18–21). These positive contrast sequences allow for the generation of positive MR signal in volumes or regions containing iron particles. The current positive contrast sequences are classified as: off-resonance methods that shift the excitation frequency to match the frequency shift caused by the iron particles (19); selective RF pulse methods, such as the inversion-recovery with oxide nanoparticles–resonant water suppression (IRON) sequence, that selectively excite regions containing iron particles (21); dephased methods that use either the shift in k space caused by the presence of iron particles or rephased areas where particles are present (18,20); and ultrashort echo time methods. The utility of the individual positive contrast methods may be application-dependent, because the sensitivity will depend on the compartmentalization and magnetic field distribution of the iron particles (22). As a result, some techniques may be better at detecting low concentrations of highly compartmentalized iron (dephasing methods), and others may be more sensitive for detecting larger field distributions. Currently a single study that compares the various methodology of detection of iron in macrophages is lacking in the literature.

In this issue of the *Journal*, Korosoglou et al. (23) present their study using IRON MRI with monocrystalline iron oxide nanoparticles (MION)-47 to detect macrophage-rich atherosclerotic plaque in a rabbit model of atherosclerosis. The investigators previously employed IRON MRI for in vivo positive contrast imaging of SPIO-loaded stem cells to localize the cells following injection on post-contrast imaging (21). In this study, pre-contrast imaging was performed in 7 Watanabe rabbits and 4 control New Zealand rabbits. A commercially available iron oxide contrast agent, MION-47, was then injected intravenously and post-contrast imaging was repeated on days 1 and 3. A second injection was performed on day 3 following imaging and post-contrast imaging was again performed on day 6. There was a significant

increase in signal intensity in aortic atherosclerotic plaque following administration of MION-47 (48% increase on day 3 and 72% increase on day 6), but no enhancement was seen in control rabbits that lack atherosclerosis. Additionally, positive contrast corresponded to the deposition of superparamagnetic nanoparticles in macrophage-rich atherosclerotic plaques. These findings are significant as they not only validate MION-47 as a successful imaging agent for macrophage-rich atherosclerosis, but also suggest that positive contrast IRON MRI can be applied to the general class of iron oxide particles. This is significant as iron oxide–based contrast agents have been previously studied in humans (13), enabling IRON MRI sequences to be directly applied to patient care. Comparison between previous findings using T_2- and T_2^*-weighted imaging with positive contrast protocols will now be necessary to validate that previously used doses of ultrasmall SPIO will be adequate for detection of atherosclerosis. However, because both methods use the same local magnetic field inhomogeneities to produce MR signal gain (off-resonance signal by IRON) or signal loss (dephasing by conventional gradient echo imaging), the correlations are expected to be similar. The specificity of the IRON technique to macrophages loaded with iron oxides needs to be established. This is critical because positive enhancement is observed in other regions outside of the arterial vessel wall. The positive contrast in these regions appears to occur at membrane interfaces and areas that may experience other sources of field inhomogeneities.

Though the findings of this report have many exciting implications for the field of MRI, it is important to recognize several limitations of this study. First, the amount of MION-47 used in this study was 10 times above the recommended clinical dosing. Additionally, to increase intraplaque macrophage uptake (saturation of the reticular endothelial system), the investigators employed 2 separate administrations of MION-47. Even though the rabbit aorta is similar in size to human coronary arteries, several limitations remain in human coronary imaging, including image resolution and the need for breath holding and electrocardiographic gating.

Despite these limitations, we eagerly look forward to the continued application of IRON MRI and other positive contrast imaging sequences for the evaluation of macrophage-rich human atherosclerosis.

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REFERENCES

1. Ambrose JA, Tannenbaum MA, Alexopoulos D, et al. Angiographic progression of coronary artery disease and the development of myocardial infarction. J Am Coll Cardiol 1988;12:56–62.
2. Little WC, Constantinescu M, Applegate RJ, et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? Circulation 1988;78:1157–66.
3. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: part I: evolving concepts. J Am Coll Cardiol 2005;46:937–54.
4. Kannel WB. Some lessons in cardiovascular epidemiology from Framingham. Am J Cardiol 1976;37:269–82.
5. Lipinski MJ, Fuster V, Fisher EA, Fayad ZA. Technology insight: targeting of biological molecules for evaluation of high-risk atherosclerotic plaques with magnetic resonance imaging. Nat Clin Pract Cardiovasc Med 2004;1:48–55.
6. Fuster V, Fayad ZA, Moreno PR, Poon M, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: part II: approaches by noninvasive computed tomographic/magnetic resonance imaging. J Am Coll Cardiol 2005;46:1209–18.
7. Jaffer FA, Libby P, Weissleder R. Molecular imaging of cardiovascular disease. Circulation 2007;116:1052–61.
8. Lipinski MJ, Frias JC, Fayad ZA. Advances in detection and characterization of atherosclerosis using contrast agents targeting the macrophage. J Nucl Cardiol 2006;13:699–709.
9. Amirbekian V, Lipinski MJ, Briley-Saebo KC, et al. Detecting and assessing macrophages in vivo to evaluate atherosclerosis noninvasively using molecular MRI. Proc Natl Acad Sci U S A 2007;104:961–6.
10. Weissleder R, Elizondo G, Wittenberg J, Rabito C, Bengele H, Josephson L. Ultrasmall superparamagnetic iron oxide: characterization of a new class of contrast agents for MR imaging. Radiology 1990;175:489–93.
11. Nahrendorf M, Sosnovik DE, Waterman P, et al. Dual channel optical tomographic imaging of leukocyte recruitment and protease activity in the healing myocardial infarct. Circ Res 2007;100:1218–25.
12. Briley-Saebo KC, Mani V, Hyafil F, Cornily JC, Fayad ZA. Fractionated Feridex and positive contrast: in vivo MR imaging of atherosclerosis. Magn Reson Med 2008;59:721–30.
13. Trivedi RA, Mallawarachi C, U-King-Im JM, et al. Identifying inflamed carotid plaques using in vivo USPIO-enhanced MR imaging to label plaque macrophages. Arterioscler Thromb Vasc Biol 2006;26:1601–6.
14. Muller RN, Gillis P, Moiny F, Roch A. Transverse relaxivity of particulate MRI contrast media: from theories to experiments. Magn Reson Med 1991;22:178–82, discussion 195–6.
15. McAteer MA, Schneider JE, Ali ZA, et al. Magnetic resonance imaging of endothelial adhesion molecules in mouse atherosclerosis using dual-targeted microparticles of iron oxide. Arterioscler Thromb Vasc Biol 2008;28:77–83.
16. Amsalem Y, Mardor Y, Feinberg MS, et al. Iron-oxide labeling and outcome of transplanted mesenchymal stem cells in the infarcted myocardium. Circulation 2007;116:I38–45.
17. Litovsky S, Madjid M, Zarrabi A, Casscells SW, Willerson JT, Naghavi M. Superparamagnetic iron oxide-based method for quantifying recruitment of monocytes to mouse atherosclerotic lesions in vivo: enhancement by tissue necrosis factor-alpha, interleukin-1beta, and interferon-gamma. Circulation 2003;107:1545–9.
18. Seppenwoolde JH, Viergever MA, Bakker CJ. Passive tracking exploiting local signal conservation: the white marker phenomenon. Magn Reson Med 2003;50:784–90.
19. Cunningham CH, Arai T, Yang PC, McConnell MV, Pauly JM, Conolly SM. Positive contrast magnetic resonance imaging of cells labeled with magnetic nanoparticles. Magn Reson Med 2005;53:999–1005.
20. Mani V, Briley-Saebo KC, Itskovich VV, Samber DD, Fayad ZA. Gradient echo acquisition for superparamagnetic particles with positive contrast (GRASP): sequence characterization in membrane and glass superparamagnetic iron oxide phantoms at 1.5T and 3T. Magn Reson Med 2006;55:126–35.
21. Stuber M, Gilson WD, Schar M, et al. Positive contrast visualization of iron oxide-labeled stem cells using inversion-recovery with ON-resonant water suppression (IRON). Magn Reson Med 2007;58:1072–7.
22. Briley-Saebo KC, Mulder WJ, Mani V, et al. Magnetic resonance imaging of vulnerable atherosclerotic plaques: current imaging strategies and molecular imaging probes. J Magn Reson Imaging 2007;26:460–79.
23. Korosoglou G, Weiss RG, Kedziorek DA, et al. Noninvasive detection of macrophage-rich atherosclerotic plaque in hyperlipidemic rabbits using "positive contrast" magnetic resonance imaging. J Am Coll Cardiol 2008;52:483–91.

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